Original article

The impact of roasting on the ochratoxin A content of coffee

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Summary Roasting coffee led to a drop in the ochratoxin A (OTA) concentration, as measured by the reference method, especially for dark type roasts. The way the beverage was prepared also affected the OTA content, which could paradoxically be higher than that of the initial roasted coffee. Assays on the thermal stability of pure OTA showed that it ought to be found in larger quantities in roasted coffee. This suggested that OTA was masked by reactions with the substrate during roasting. The absence of OTA in green coffee is therefore the best guarantee of safety.

Keywords Coffee process, ochratoxin A in coffee beverage, ochratoxin A thermal destruction.

Introduction

Ochratoxin A (OTA) is a secondary metabolite that is produced by some moulds of the genera *Aspergillus* and *Penicillium* with nephrotoxic, immunotoxic, teratogenic and carcinogenic effects (Höhler, 1998; Pfohl-Leszkowicz & Castegnaro, 1999). There are three main moulds that produce OTA that have been found in association with coffee: *Aspergillus niger*, *A. carbonarius* and *A. ochraceus* (Frank, 2001; Joosten *et al.*, 2001). OTA is found at different stages of coffee production and processing: cherry, green coffee, roasted coffee (Viani, 1996; Patel *et al.*, 1997; Stegen *et al.*, 1997; Mantle & Chow, 2000; Romani *et al.*, 2000; Taniwaki *et al.*, 2003).

Roasting is a heat treatment that can have an effect on OTA content. Prior to 1988, OTA was thought to be broken down during roasting but, since then, numerous experiments have been published giving sometimes contradictory results; some studies reported strong OTA destruction after roasting (Viani, 1996; Blanc *et al.*, 1998; Stegen *et al.*, 2001), whilst others only reported a slight reduction after heat treatment of naturally and artificially contaminated batches of green coffee (Tsubouchi *et al.*, 1987; Studer-Rohr *et al.*, 1995). Transfer of the toxin into the beverage during brewing has also been tested with contradictory results ranging from 0 to 133% (Tsubouchi *et al.*, 1987; Micco *et al.*, 1989; Studer-Rohr *et al.*, 1995; Stegen *et al.*, 1997; Leoni *et al.*, 2000). These contradictions were attributed to variability in the types of coffee contamination, different roasting conditions and heterogeneity in mycotoxin contamination (Stegen *et al.*, 2001). The problem of OTA resistance to technological processes, notably heat treatment, has been studied on other products: moisture had an effect on OTA destruction in wheat; there was no change after 40–160 min of dry heat treatment, whereas there was a 50% reduction after 120 min of humid heat treatment (Boudra *et al.*, 1995).

The purpose of our work was to investigate the effect of roasting on destruction kinetics and OTA

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content in arabica coffee from Mexico, along with the effect of the brewing method, i.e. extraction, on OTA content in the coffee beverage.

Material and methods

Coffee

The batches of green arabica type coffee (*Coffea arabica)* came from the Coatepec region, Veracruz region, Mexico (2001–02 harvest).

Fungus strain

We isolated a strain which produced OTA from parchment coffee from the Coatepec region: *A. ochraceus* Wilhelm (MULC 44640). The strain was deposited in the BCCM^{TM}/MULC Culture Collection (Leuven Catholic University, B-1348 Louvain-la-Neuve, Belgium).

Inoculum preparation and green coffee contamination techniques

Aspergillus ochraceus was grown on potato dextrose agar medium, pH 3.5 at 25 °C for 5 days. The spores were collected by scraping the surface of one plate in 100 mL of a 0.04% Tween-80 solution. After counting on a haematocytometer, 75 mL of the suspension was used to inoculate 1500 g of green coffee sterilized in the autoclave for 20 min at 121 °C. The culture was incubated at 28 °C for 15 days.

Roasting techniques and coffee sampling

Samples (100 g) were roasted in a Probat-Werne type RE1 pilot roaster. Two temperatures were used, 200 and 250 °C, and three different degrees of roasting – light, medium and dark – were obtained depending on the length of time spent by the coffee in the roaster.

Bean swell, weight loss and colour were used to assess the degree of roasting. Colour was assessed with a colorimeter (Chroma Meter CR-200 Minolta, Konica-Minolta France, Roissy, France) using the CIELAB colorimetric equations system, which gave the dimensions L^*, a^* b^* used to characterize the colour of the analysed sample. Table 1 gives the characteristics of the different roasts.

Table 1 Roasting conditions

Roasting degree	Roasting air temp. (°C)	Roasting time (min)	Colour value ground coffee (*L** value)
Light	200	4.4	32.0 ± 2.6
	250	2.8	30.2 ± 0.3
Medium	200	4.9	25.3 ± 1.2
	250	3.0	25.9 ± 2.6
Dark	200	5.3	22.9 ± 0.6
	250	3.3	23.1 ± 1.0

Kinetics of OTA destruction on coffee

A 100-g sample of contaminated coffee was placed in the roaster and at a given time, a 10-g sample was removed (three replicates). Each sample was then extracted, purified on an immunoaffinity column, and analysed by HPLC. The kinetics temperatures chosen corresponded to those used for roasting of the contaminated coffee: 200 and 250 °C.

Beverage preparation techniques

The beverages were prepared from the sample that had remained the most contaminated after roasting. We then assessed whether or not there was any loss of toxin in the beverage prepared by the three most commonly used methods: 'filter' coffee (15 g of ground coffee in 250 mL of boiling water), 'espresso' coffee (7 g of ground coffee in 30 mL of water) and 'plunger' coffee (brewing for 5 min; 50 g of ground coffee in 1 L of boiling water) (Clarke & Vitzthum, 2001).

OTA quantification

In green coffee

Coffee samples were frozen at −80 °C then ground to pass a 0.5 mm sieve and analysed for OTA (Nakajima *et al.*, 1997). The samples (10 g) were extracted for 30 min with 100 mL of methanol/3% sodium bicarbonate (50:50), the extracts (10 mL) were filtered and diluted with 30 mL of phosphate-buffered saline and applied to an immunoaffinity column (Ochraprep®, Rhône Diagnostics, Glasgow, UK). OTA was eluted with 3 mL HPLC grade methanol. The eluate was evaporated to dryness under a stream of nitrogen at 70 °C, and the residue was redissolved in 1 mL

of HPLC mobile phase and then quantified by HPLC (Shimadzu LC-10ADVP, Japan, with fluorescence detector). The mobile phase consisted of distilled water/acetonitrile/glacial acetic acid (51:48:1). The injection volume was 100 μL and the flow rate was 1 mL min^{-1}. OTA was detected by absorption at 333 nm excitation and 460 nm emission, and a retention time of 13.3–13.5 min. A standard curve of OTA was established from an ochratoxin standard (1000 ng mL^{-1}; ref PD 226 R. Biopharm Rhône Ltd, Glasgow, UK): the limit of detection was 0.075 ng mL^{-1}.

In roasted coffee

Ten grams of ground roasted coffee were extracted for 30 min with 100 mL of 3% methanol/bicarbonate solution (20/80); 5 mL of the filtered extract were diluted with 40 mL of PBS buffer and cleaned through an immunoaffinity column (Pittet *et al*., 1996). The analysis then proceeded as for green coffee.

In the beverage

Ten millilitres of coffee beverage were diluted in 40 mL of PBS and cleaned through an immunoaffinity column. OTA was quantified in 1 mL of concentrated extract under the same conditions as those described for green coffee.

On filter paper

Ten microlitres of a methanol/water (3/7) solution at 200 ng mL^{-1} of pure OTA (from the standard solution used above) were deposited on filter paper discs placed in an oven at different temperatures (150, 200 and 250 °C). The sampling times were chosen for each of these temperatures in accordance with the actual roasting times used for a contaminated coffee. On leaving the oven, each disc was placed in a vial to which 1mL of HPLC solvent was added. All the experiments and assays were duplicated.

Results and discussion

Effects of roasting on green coffee naturally contaminated with OTA

We subjected green coffee containing 0.4 μg kg^{-1} of OTA to three types of roasting: light, medium and dark. The OTA disappeared in each case (detection limit of the method: 0.1 μg kg^{-1}). The OTA content of the green coffee used in this experiment was not necessarily representative of what might usually be encountered; indeed, concentrations of 0.4–80 μg kg^{-1} are common (Micco *et al*., 1989; Studer-Rohr *et al*., 1995; Otteneder & Majerus, 2001). Consequently, it was necessary to carry out tests on green coffee containing higher quantities, obtained by artificial contamination.

Effects of roasting on a green coffee artificially contaminated with OTA

We did two contamination experiments with an *A. ochraceus* culture. After 15 and 8 days of contact, we obtained green coffees containing 53.2 and 16.6 μg kg^{-1} of OTA respectively. These two coffees were subjected to the three types of roasting and two temperatures. It could be seen (Table 2) that OTA loss increased in line with the degree of roasting. Rather curiously, it was found that for a given degree of roasting, estimated by luminance, the higher temperature did not lead to the maximum loss. This might be explained by the roasting time necessary (Table 1). In these experiments, except in one case, the OTA content of

Table 2 Average ochratoxin A (μg kg^{-1}) contents before and after several roasting degrees of green coffee inoculated with *Aspergillus ochraceus*

	Light		Medium		Dark	
Roasting degree temp.	**200 °C**	**250 °C**	**200 °C**	**250 °C**	**200 °C**	**250 °C**
Coffee 1 53.2 ± 0.3						
OTA residual	21.9 ± 0.3	25.2 ± 0.1	11.6 ± 1.1	12 ± 0.1	9 ± 0.1	10.6 ± 0.1
OTA reduction %	58.7	52.6	78.3	77.4	82.9	80.1
Coffee 2 16.6 ± 0.2						
OTA residual	n.t.	13.7 ± 0.2	n.t.	5.9 ± 0.1	n.t.	2.3 ± 0.3
OTA reduction %		17.4		64.5		85.7

n.t., not tested.

roasted coffee remained over 3 μg kg^{-1}, the concentration specified in European legislation (Romani *et al.*, 2000). With these initial OTA concentrations, roasting did not generally reduce OTA levels in the coffee to a level to conform with legislation or advisory limits.

Effect of brewing on OTA content in the beverage

Using roasted coffee containing 25 μg kg^{-1} of OTA, we prepared a coffee beverage by the 'filter', 'espresso' and 'plunger' techniques, then quantified the resulting OTA content (Table 3). We did this using the beverage directly as the extraction product.

Curiously the OTA content, extrapolated to 1 kg of coffee, was sometimes higher in the beverage. This had already been reported (Studer-Rohr *et al.*, 1995) and could be explained by the particularity of the extraction which, in this case, was carried out hot when the beverage was prepared. We also found that espresso preparation, where the time spent in contact with water is short, did not display this phenomenon. The increase sometimes seen could also be explained by 'masking' of the OTA molecule during roasting. To check the first hypothesis, we compared the method of cold extraction from roasted coffee proposed by Pittet *et al.* (1996) used in this work with a hot variant (reflux heating up to boiling for 10 min). It is noteworthy that the AOAC method (Entwisle *et al.*, 2001) also uses a cold extraction. Hot extraction gave an OTA concentration of 30.7 ± 0.2 μg kg^{-1}, whereas cold extraction gave 21.2 ± 0.3 μg kg^{-1}. This might explain the increase sometimes observed. It is therefore reasonable to question the validity of the method used in terms of absolute determination of OTA content. To test the second hypothesis, it would have been necessary to do an in-depth study which is beyond the scope of this work.

Table 3 Effect of brewing on ochratoxin A (OTA) content in the beverage

	Roast and ground coffee	'Filter' coffee	'Plunger' coffee	'Espresso' coffee
OTA (μg kg^{-1})	24.7 ± 0.1	30.1 ± 0.1	30.1 ± 1.7	18.4 ± 0.7
% Recovery	100	122	122	75

Study on the kinetics of OTA disappearance during roasting

We studied changes in OTA content during two roasting operations at 200 and 250 °C. Figure 1 gives the results of the experiment. The $\ln C/C_o$ representation as a function of time revealed two phases: slow destruction followed by rapid destruction. The values of the slopes obtained for the first phase were −0.07 for 200 °C and −0.18 for 250 °C.

Study on the kinetics of OTA disappearance during heating

In order to reveal any interaction in the substrate during roasting, we studied the effect of heat on pure OTA over time (Fig. 2).

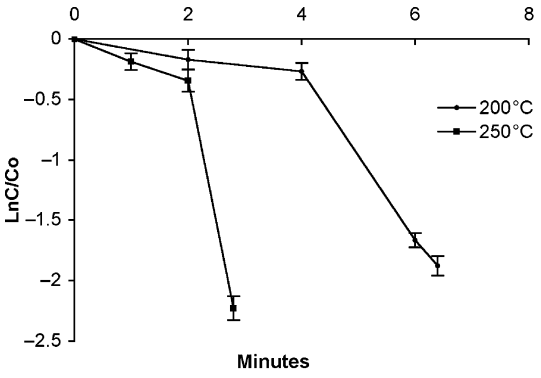

Figure 1 Kinetics of ochratoxin A disappearance during roasting.

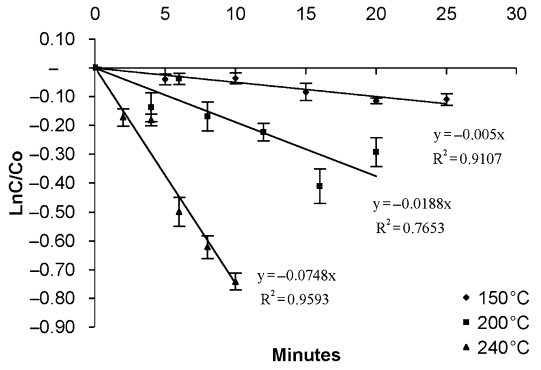

Figure 2 Kinetics of pure ochratoxin A disappearance during heating.

The results obtained revealed first-order reaction in this case (one phase only). The k coefficients that were obtained were weaker than those obtained in the first phase at the same temperature on coffee substrate (from −0.005 to −0.075); however, the magnitude was comparable. These results suggest the existence of reactions that 'masked' OTA during roasting, and such masking probably involved several types of reactions, which explained why the reaction on coffee was not a first order. OTA could be bound to coffee components. It is known, for example, that OTA binds to the plasma proteins (Il'ichev *et al.*, 2002) and coffee does contain proteins. Changes to OTA during heat treatment have also already been described; at high temperatures, a partial isomerization of OTA in position C3 into a diastereomer occurred (Studer-Rohr *et al.*, 1995). When the chromatograms obtained during pure OTA heating (Fig. 3a,b) were examined, it was found that in parallel with OTA disappearance, there was a 'proportional' appearance of a peak corresponding to a new product that had been formed (which did not occur in a heated control without OTA). It may be that this was a product of destruction, but may also be a product of rearrangement or polymerization. Consequently, we prefer to speak of OTA disappearance rather than destruction.

At this stage, we felt it would be worth searching for the existence of that product in roasted coffee (Fig. 3c,d); it was never possible to detect it. As quantification from roasted coffee involved cleaning by immunoaffinity, it may be that the product was not recognized by the anti-OTA antibody. In any event, this phenomenon did not explain the difference between the apparent efficiency of roasting and that of heat treatment on pure OTA. It is likely that other reactions occur with OTA in coffee. If those reactions occur earlier than those observed with pure OTA, it may explain the absence of the peak described above, although it corresponded to an antigenic product, which is possible if it were a dimer. These hypotheses may help in explaining the phenomena described in the coffee beverage.

Consequently, OTA disappearance, measured by the reference method, did not necessarily mean an absence of toxic risk, as the transformation or combination products may be just as dangerous as OTA. A similar situation is known; aflatoxin B treated with ammonia gives aflatoxin D1 (Grove *et al.*, 1984). This shows the need for further chemical and toxicology work to really ascertain the impact of roasting.

Conclusion

The effect of coffee roasting on its ochratoxin content remains a controversial subject. Whilst the ochratoxin content quantified by the reference

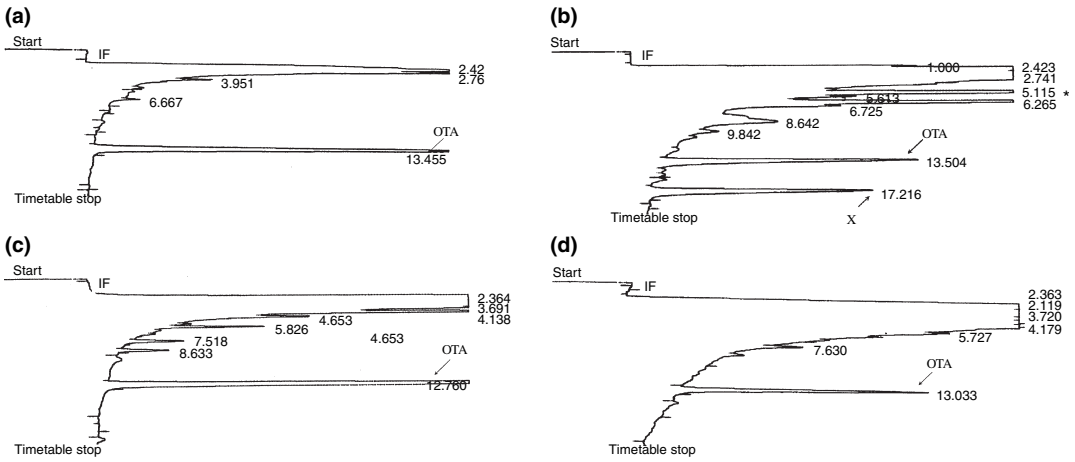

Figure 3 HPLC chromatograms of pure ochratoxin A (a) before and (b) after heating 2 min at 250 °C and HPLC chromatograms of same coffee samples (c) before and (d) after roasting 3 min at 250 °C. *These modifications corresponded to the heating of the paper support (a reference was made; result not shown).

method decreased, in not insubstantial proportions for some treatments such as dark roasting, giving values that complied with the legislation, it is not sure that this operation makes the beverage harmless. Indeed, depending on how the beverage was prepared, its content could be higher than that expected from the content measured in the roasted coffee. Moreover, given the relative heat stability of OTA, it is likely that its content primarily decreases because of the formation of compounds linked chemically to the nature of the substrate. For instance, despite the apparently positive effect of roasting, the toxicological problem may remain. It is therefore preferable not to count on the effect of roasting to reduce OTA content, but rather to give priority to obtaining green coffee with a low or zero content.

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